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The crystal structure of CFTR, the chloride ion channel defective in cystic fibrosis, has not yet been solved. Here we present two new homology models of human CFTR based on template structures from related ABC transporters. The first is based on the bacterial transporter Sav1866 and is representative of the open channel state of CFTR. Unlike previous Sav1866-based homology models of CFTR, our model incorporates several key structural features expected from experiment, including the proper positioning of pore-lining residues and important salt bridges. The second is based on the crystal structure of murine P-glycoprotein and models the closed state of CFTR. We performed targeted molecular dynamics simulations using these two models as end states, in order to gain insight into the conformational changes that CFTR undergoes during its gating cycle. Our simulations reveal that CFTR gating involves a conformational wave that is initiated at the nucleotide-binding domains, and propagates through interactions in the intracellular loops to the membrane-spanning domains. Analysis of our simulations also led to a better understanding of the relative motions of the twelve transmembrane helices in CFTR, and how they alter pore structure during gating. Our MD simulations allowed identification of key inter-residue interactions that stabilize the end states as well as transient interactions that may exist in the intermediate stages in gating. Charting the progression of these interactions provides a timeline of events likely to occur during gating, and may prove invaluable in furthering our understanding of structure-function relationships in CFTR. Finally, we report on preliminary simulations of chloride ion conduction in the open CFTR channel, which reveal key interactions of pore-lining residues with passing ions, as well as identifying the putative narrow region in the pore that may form a selectivity filter in this channel. (NIH-2R56DK056481-07)

3213-Pos Board B368
The Structural and Functional Imporatnce of Type II Divergent Amino Acids in the Cystic Fibrosis Transmembrane Conductance Regulator
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The cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ancient ABC transporter superfamily. Most ABC proteins are transporters that operate via an alternating access mechanism, except for CFTR which is best known for its activity as a chloride channel. We compared CFTR protein sequences to those of its closest mammalian paralogs, ABCC4 and ABCC5, and used the statistical analysis program DIVERGE to analyze a CFTR-ABCC4-ABCC5 multiple sequence alignment to identify residues (called Type II divergent residues) most likely to be involved in the evolutionary transition from transporter to channel.The Type II divergent amino acids were further divided into three groups based on their functional differences. Group I: charged residues that contribute to maintaining CFTR's open pore architecture, including R117, R334, and R352. CFTR channels with mutations at these sites exhibited multiple open states with significantly shorter burst durations compared to WT-CFTR. Group II: sites where mutation resulted in no detectable current in *Xenopus* oocytes and loss of surface expression in HEK293 cells, including P140. Group III: sites where mutation did not alter single channel behavior or block by glibenclamide, including N187, T262, Q1038, K1060, N1148. These data suggest that Type II divergent amino acids may be key players in CFTR channel activity, at least in part by converting the conformational changes of a transporter into an open permeation pathway with a stable open state. This analysis sets the stage for understanding the evolutionary and functional relationships that make CFTR a unique ABC superfamily protein. (NIH-2R56DK056481-07)

3214-Pos Board B369
Molecular Models of the Closed State of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
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Cystic fibrosis is a monogenic genetic disease due to mutations in the gene coding for a membrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR controls the flow of chloride ions through the apical membrane of epithelia, thus regulating the transepithelial movement of both water and ions, needed for the production of healthy secretions. Mutations in the CFTR gene affect the proper folding, trafficking and function of CFTR, resulting in the formation of thick and viscous mucus that accumulates in different organs, notably in the lungs where it predisposes to persistent bacterial infections.
CFTR belongs to the ATP binding cassette (ABC) transporter superfamily. Human ABC transporters share a common structural architecture, which minimally consists of two transmembrane domains (TMDs), forming in CFTR the pore for chloride flow, and two nucleotide binding domains (NBDs), for ATP binding and hydrolysis.
The lack of crystal structures hampers a global understanding of the structure and function of CFTR, and thus the development of approaches directly targeting defective CFTR. Here, we present molecular models of CFTR in different conformational states, built on available structural data. We focus in particular on closed state conformations, and on the interactions at the NBD interface. The models are used, together with available experimental data, to infer the roles played by specific residues in the gating transitions, allowing hypothesis testing through mutagenesis and functional studies.

3215-Pos Board B370
Structural Basis for the State-Dependent Reactivity of Engineered Cysteines in the Pore of the CFTR Channel
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The cystic fibrosis transmembrane conductance regulator (CFTR) is the only known member of the ATP binding cassette transporter superfamily that functions as an ion channel. Its architecture is analogous to that of ABC exporters such as p-glycoprotein, MsbA and Sav1866, which export their substrates using an alternating access mechanism driven by catalytic cycles coupled to ATP hydrolysis. The opening and closing of the CFTR channel is also driven by ATP catalytic cycles. We studied the state-dependence of the reactivity of three engineered cysteines located at positions 334, 337 and 338 in transmembrane helix 6 (TM6) of CFTR toward externally-applied, thiol-directed reagents, in order to investigate the conformational changes associated with channel gating. The engineered cysteine at position 334 was more reactive in the closed state, consistent with the findings of Zhang et al. (2005; J. Biol. Chem. 280: 41997-42003), while the engineered cysteines at positions 337 and 338 were more reactive in the open state. These findings are consistent with the predictions of homology models of the CFTR channel based on the crystal structures of bacterial homologues captured in inward- and outward-facing states of the proteins. Funded by the National Institute of Diabetes and Digestive and Kidney Diseases, the Cystic Fibrosis Foundation, and the American Lung Association.

3216-Pos Board B371
Probing the CFTR Chloride Channel with Channel Permeant Thio-Reactive Reagent Au(CN)2-
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Previous cysteine scanning experiments on the CFTR channel have identified transmembrane segments (TM) 1, 3, 6, 9, and 12 as pore-lining components. using bulky MTS reagents, we and others have demonstrated a restrictive region in TM 1 and 6 that prevents MTS from passing through the CFTR pore. State-dependent modification data also suggest a lack of a physical gate cytoplasmic to this restrictive region. Here we further tested gated access of engineered cysteines in TM6 by using channel permeant probes, like [Ag(CN)2]- and [Au(CN)2]-. We first focused our effort on two positions, 338 and 344, located external and internal respectively to the restrictive region. In excised inside-out patches, application of [Au(CN)2]- causes a biphasic decay of the currents for both I344C- and T338C-CFTR. Since the fast phase is reversible and also seen with the cysless background, it is likely the slow phase reflects covalent modification of introduced cysteines by [Au(CN)2]- while the fast phase is due to blockade of the pore by the negatively charged reagent. Measurements of the modification rate in the presence or absence of ATP suggest that I344C can be accessed in both the open and the closed states, consistent with our previous studies using channel impermeant probes. However, preliminary data suggest the modification rate for C338 in the presence of ATP is ~10-fold higher than that in the absence of ATP. More extensive studies are in process to unravel the underlying gating motion of CFTR's TMs.

3217-Pos Board B372
Seeing ATP Hydrolysis in Real Time, What does it Tell Us?
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CFTR, a chloride channel evolved from the exporter member of the ATP binding cassette (ABC) Protein Superfamily, is gated by association and dissociation of its two NBDs. Single-channel data have led to a gating model with one-to-one stoichiometry between the gating cycle and the ATP hydrolysis